(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 340 793 A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 89108162.2

(22) Date of filing: 05.05.89

(51) Int. Cl.[4]: C12N 15/00 , A61K 39/00 , A61K 35/26

(30) Priority: 04.05.88 IL 86278

(43) Date of publication of application: 08.11.89 Bulletin 89/45

(84) Designated Contracting States: AT BE CH DE ES FR GB GR IT LI LU NL SE

(71) Applicant: YEDA RESEARCH & DEVELOPMENT COMPANY, LIMITED
P.O. Box 95
Rehovot 76100(IL)

(72) Inventor: Eshhar, Zelig
18, Hess Street
Rehovot(IL)
Inventor: Gross, Gideon
Moshav Almagor
Doar Korazin 12340(IL)
Inventor: Waks, Tova
Keren Kayemet 12
Petah Tikva(IL)

(74) Representative: Vossius & Partner
Siebertstrasse 4 P.O. Box 86 07 67
D-8000 München 86(DE)

(54) **Endowing cells with antibody specificity.**

(57) There are produced recombinant gene pairs which endow mononuclear cells, mainly various lymphocyte type cells, with antibody-type specificity. In specific gene pairs the rearranged gene pairs code for a binding site of an antibody molecule from the same species, of the T-cell receptor gene, or another species. Gene pairs of the invention code, for example for antibodies specific towards tumor-specific antigens, viral antigens, modified self antigens, bacterial or fungal antigens, autoimmune type disease antigens and the like.

The invention further relates to expression vectors for the effective transfection of such cell types comprising such a recombinant gene pair, to methods for producing same and to pharmaceutical compositions comprising as active ingredient an effective quantity of lymphocytes transfected with such gene pairs.

EP 0 340 793 A2

ENDOWING CELLS WITH ANTIBODY SPECIFICITY

The invention relates to gene pairs of recombinant DNA, which gene pairs are adapated to endow mononuclear cells with antibody-type specificity. Various types of cells are suitable, for example lymphocytes such as T-killer cells, T-helper cells, T-suppressor cells, lymphokine activated cells and natural killer cells.

The gene pair consists of one gene which comprises genomic segments containing exons coding for the constant region of the cell receptor, and another gene which comprises gene segments containing the leader and rearranged variable and joining exons encoding a specific antibody's heavy and light chains.

The invention further relates to suitable vectors for transfecting cells of the type defined above with such gene pairs. The cells may be transfected with a single vector bearing the gene pair, or with two different vectors, each bearing one gene of the gene pairs.

The invention further relates to cells of the type defined above into which such gene pairs have been introduced so as to obtain their expression, and also to pharmaceutical prophylactic and curative compositions containing an effective quantity of such cells.

In general terms, the invention relates to a process for the generation of lymphocytes transfected with one or two expression vectors containing a chimeric T-cell receptor gene. As set out in the following there was constructed a model system which comprises expression vectors which were transfected and which were functionally expressed in T-killer cells.

Because of the restrictions imposed by corecognition of self MHC plus antigen, the acquisition of new specificity by grafting of TcR genes is limited to experimentations either in vitro or in vivo in inbred combinations. Such manipulations are practically impossible in an outbred population.

Unlike antibodies, the T cells recognize antigens only in association with products of the major histocompatibility complex (MHC). Such dual recognition is mediated by combination of the variable regions of both the α and β chains that comprise the TcR. Recently it became possible to endow T cells with a given specificity by DNA mediated transfer of cloned genes coding for the α and β TcR chains (Dembic et al. Nature 320 232-238 (1986)). In general, the expression as a dimer of both α and β chains of a given TcR has been required in order to display a defined specificity although it has been implicated that the $V\beta$ is responsible for the MHC specificity (Saito et al. Nature 329 256-259 (1987)).

In order to overcome the limitations set out above, and in order to be able to design at will T-cells having a desired predetermined specificty, there were constructed T-cells with antibody-typ specificity. The invention is applicable to a wide variety of cells as set out herein.

Based on the extensive degree of similarity in structure and organization between the antibody and TcR molecules, it was assumed that it will be possible to replace the V-region of TcR with an antibody's one in a manner that will result in a functional chimeric TcR. According to the invention it is possible to construct and functionally express chimeric TcR that recognize antigen in non-MHC restricted manner and effectively transmit transmembrane signal for T cell activation.

According to the invention there were constructed chimeric T cell receptor genes by recombining the V_H and V_L gene segments of an anti-TNP antibody with the constant region exons of the T cell receptor's (TcR) α and β chain. Following transfection into cytotoxic hybridomas, expression of a novel functional T cell receptor was detected. The chimeric receptor manifested the idiotype of the antibody and endowed the T cells with non-MHC restricted, anti-TNP reactivity. This mod l system demonstrates that chimeric TcR with an antibody-like binding site can be designed and functionally expressed in a variety of T-cells. This manipulation offers a novel approach for engineering at will T-cells of any desired specificity, provided that such specificity can be predefined by monoclonal antibodies. The various aspects of this invention are suitable gene pairs for introduction and expression in certain vectors, the use of such vectors for transfecting cells (T-cells and others, as defined) in order to enhance them with a predetermined antigenic specificity. The invention further relates to pharmaceutical compositions for the prevention and cure of certain diseases. Other aspects will become clear hereinafter.

As stated above the invention relates to lymphocytes, comprising populations of T-killer cells, T-helper cells, T-suppressor cells, lymphokine activated cells, natural killer cells and the like.

As stated above the invention relates to a recombinant gene-pair adapted to endow mononuclear cells with antibody-type specificity, wh re the genes are:

a. gene segments containing exons coding for the constant region of the T-cell receptor (α, β, γ, δ,chains),

b. gene-segments containing the leader and rearranged variable + joining exons encoding for a specific antibody's heavy and light chains, the said

mononuclear cells being lymphocytes, comprising populations of T-killer cells, T-helper cells, T-suppressor cells, lymphokine activated cells, natural killer cells, and the like.

The above gene segments can be genomic or cDNA segments.

According to a preferred aspect the invention relates to a gene pair with gene segments coding for the constant region of α or β chains, each of which being ligated with either the rearranged variable gene segment of the antibody's heavy or light chain and vice versa, it being clear that the T-cell receptor can be of the same species or that of different species. Preferably the rearranged gene segments code for a binding site of an antibody molecule either from the same species of the T-cell receptor gene or another species where the rearranged gene segments code for antibody specific towards tumor-specific antigens, tumor-associated antigens, viral antigens, modified self-antigens, parasitic antigens, bacterial antigens, fungal antigens, autoimmune disease type antigens, or other foreign antigens. According to a further embodiment the rearranged gene segments code for monoclonal antibodies reacting with a defined type of tumor cells. The invention further relates to expression vectors for the efficient transfection of a cell type, comprising a recombinant gene pair as defined above. Such a vector can be a plasmid backbone containing promoter, polyadenylation site and drug selection markers. Preferred vectors according to the invention are the plasmids pRSV2 and pRSV3. There is preferably used a pair of expression vectors in which one vector comprises a plasmid with one selection marker (say neo^R) into which a rearranged gene segment coding for the variable region of the antibody's light chain together with either the gene segment coding for the constant region of the T-cell receptors or β chains are cloned, and the second vector comprises another selection marker (say gpt) into which a rearranged gene segment coding for the variable region of the antibody's heavy chain together with gene segment of the constant region of the complementary T-cell receptor's chain (β or α) used in the first vector.

The invention further relates to mononuclear cells containing a chimeric pair of genes as defined above.

Yet another aspect of the invention relates to a composition for treating a patient in need thereof comprising as active ingredient lymphocytes which have been taken from th pati nt, propagated in vitro, which hav been transfect d with a gene pair as above described or with a vector as d fined above.

Yet another aspect relates to a process for producing expression vectors according to the invention and for constructing a gene pair which comprises selecting a hybridoma producing a monoclonal antibody for the desired antigen, constructing a genomic library from the restricted DNA fragments that contain the rearranged V_L and V_H exons, isolating the clones carrying full length rearranged V_L and V_H exons, isolating from them the DNA segments containing the leader exon and the rearranged VDJ_H and VJ_L exons and subcloning each of them into an expression vector containing either the neo^R or gpt selection marker, inserting into each of the same vectors, 3′ to the VDJ_H or VJ_L either one of the genomic fragments containing all of the constant region exons and 5′ flanking untranslated regions of the T-cell receptor α and β chains isolated from embryonic DNA library, resulting in a set of chimeric genes comprised of $V_HC\alpha$, $V_LC\beta$, $V_LC\alpha$, $V_HC\ \beta$ each of which is cloned in one of the expression vectors containing either the neo^R or gpt selection markers. One aspect of the invention relates to a process where a combination of two plasmids is used for the transfecton of the T-cell, one of which comprises the variable region of the light chain and the other either the constant region of α or β ; the other the variable region of the heavy chain with the other of the α- or β .

The following example is a model experiment which demonstrates the feasibility of the above general principles and its wide scope of applicability. The invention is not restricted to this specific embodiment.

Example

To construct the chimeric TcR genes we ligated genomic segments each one containing th rearranged V_J and leader exons of either heavy or light chain of the Sp6 anti-TNP, IgM antibody (Ochi et al. Proc.Natl.Acad.Sci.USA 80 6351-6355 (1983)) with constant region exons of either the α or β chains of the TcR. The chimeric genes were inserted into pRSV expression vector containing the Rous sarcoma promotor and either the Neo^R or the gpt drug resistance genes. By protoplast fusion we transfected each of the vectors into MD.45 - a CTL hybridoma of BALB/c origin that can be stimulated by H-2D^b cells both for IL-2 production and specific killing of target cells (Kaufmann et al. Proc.Natl.Acad.Sci.USA 78 2502-2507 (1981)). Out of the drug resistant transfectants we selected the hybridomas that transcribed the chimeric gen (using V_H and V_L probes). The clone producing the highest levels of ne chain, was retransfected with the construct containing the complementary chain and the other drug mark r. Doubl transfectants that grew in th presence of both mycophenolic acid and G.418 were checked by Northern analysis

for the transcription of both chimeric genes and by immunoblotting and immunoprecipitation for th expression of the Sp6 idiotype (using the 20.5 mAb). The functional expression of the chimeric receptor was evaluated by the ability of the cells to respond by IL-2 production to TNPylated cells of various origins and by TNP-protein antigens either alone or presented by different cells.

Following DNA transfer of the chimeric genes combination of either $V_LC\alpha$ + $V_HC\beta$ or $V_LC\beta$ + $V_HC\alpha$ into the MD.45 CTL hybridoma, almost all the transfectants transcribed both complementary chains of 1.8 Kb for $V_LC\alpha$ or β and 1.9 Kb for $V_HC\alpha$ and β chains (Gross et al. submitted for publication (1988)). The expression of the chimeric polypeptide was analyzed by immunoblotting of cell lysates using anti-Sp6 idiotype mAb 20.5 or by immunoprecipitation using the 20.5 mAb and anti-TcR β 8.3 subgroup F23.1 mAb (Fig. 1). Under non-reducing conditions the anti-id and anti- β TcR reacted with a broad band of apparent molecular weight of 80 Kd that is composed of two bands of 83 Kd and 77 Kd. In some transfectants a band of 42-45 Kd was also apparent. After reduction, however, the idiotypic determinant recognized by 20.5 mAb in the immunoblot was destroyed and the 80 Kd complex was dissociated into the 42 Kd polypeptide. Interestingly, transfectants that received either the $V_HC\alpha$ or $V_HC\beta$ alone also expressed the 83 Kd complex as well as the 42 Kd chain carrying the 20.5 idiotype. Considering that Md.45 hybridoma expresses its $\alpha\beta$ dimer (Becker et al. Nature 316 606-619 (1985)) and only the β chain of BW 5147 fusion partner (Lustgarten and Eshhar unpublished data), together with the fact that the 20 . 5 idiotype (as well as the anti-TNP binding site) is expressed solely on V_H Sp6 and is sensitive to denaturation under reducing conditions, we can conclude that in transfectants that had received the $V_HC\alpha$ or $V_HC\beta$ gene, a chimeric chain is expressed that can form functional dimer with the autologous complementary α or β TcR chains. The chains in part of the dimers are not linked by disulfide band and therefore migrate as single chain in SDS-PAGE. The double transfectants most likely express on their surface in addition to the $V_LC\alpha$ - $V_HC\beta$ (or $V_LC\beta$ - $V_HC\alpha$) chimeric receptor dimers, also the heterodimers that result from various combinations of pairing of the chimeric chain with the complementary autologous α and β chains. These results in the broad band observed in the immunoprecipitation surface iodinated TcR by either anti-id or anti-TcR (Fig. 1).

In order to study whether the chimeric receptor preserved th antibody's anti-TNP specificity and the ability to transmit transmembrane signal for T cell activation, we coincubated the transfectants with different stimulator cells either TNPylated or in th pres nce of various TNOP-protein antig ns. Fig. 2 shows th degree of IL-2 production by G.2-one of the transfectant that received $V_LC\beta$ + $V_HC\alpha$ chimeric chains. G.2 expressed on its surface both the autologous TcR as evidenced by its reactivity (like the parental MD.45 hybridoma) toward EL-4 stimulator cells. Unlike MD.45, G.2 underwent triggering by TNP covalently coupled to A.20 (BALB/c B lymphoma) or UC.29 (human B lymphoblastoid). In addition TNPylated proteins (such as TNP-BSA, TNP-KLH and others), could stimulate IL-2 production by G.2 either in a soluble form and even better in the presence of BALB/c spleen cells or A.20 cells that are known to be good antigen presentors. Interestingly, the transfectants that received only the $V_HC\alpha$ or the $V_HC\beta$ chimeric gene and expressed the Sp6 idiotype, could also respond to TNP indicating that the V_H of Sp6 contains all the information needed to construct the TNP-binding site.

Taken together, our results clearly demonstrate that it is possible to construct, transfect and functionally express chimeric T cell genes that manifest antibody specificity. This novel approach should be extended to enable the engineering at will of the specificity of T cells in non-MHC restricted manner, in a way that a given set of genes could b transferred to T cells of any origin. Such T cells could then be returned to their donor and manifest the acquired specificity. Following such manipulation, the cells acquire a new specificity encoded by the chimeric genes that is of antibody-type, i.e., not restricted by self-MHC molecules.

The results obtained demonstrate that in a similar manner it is possible to prepare a wide variety of pairs of such chimeric genes that are directed to various target antigens which are predefined by specific monoclonal antibodies. Such antigens can be those found in tumor cells of a certain cancer, viral antigens, modified self antigens, antigens of bacteria, parasites, fungi, antigens of autoimmune diseases, and any other antigens of which directing cellular immune responses can benefit the patient.

It is one of the advantages of this invention that it enables taking the patient's own cells, their propagation in vitro, to select (if needed) a certain effector subpopulation (killers, helper, cr suppressor cells), and to direct the desired specificity of such cells by introducing into them the pair of engineered chimeric genes. Such cells, upon reimplantation into the patient, will function against the target antigens as dictated by the chimeric genes.

each of the obtained vectors 3′ to the VDJ_H and VJ_L either one of the genomic fragments containing all of the constant region exons and 5′ flanking untranslated regions of the T-cell receptor α and β chains isolated from an embryonic DNA library, resulting in a set of chimeric genes comprised of $V_HC\alpha$, $V_LC\beta$, $V_LC\alpha$, $V_HC\beta$ each of which is cloned in one of the expression vectors containing the selection marker, preferably the neo^R or gpt selection marker.

19. A process according to claim 18, wherein a combination of two plasmids is used for the transfection of the T-cell, wherein the first plasmid comprises the rearranged variable region of the antibody's light chain in combination with either the constant region of the T-cell receptor's α or β chain, and wherein the second plasmid comprises the rearranged variable region of the antibody's heavy chain and the complementary T-cell receptor's chain (β or α).

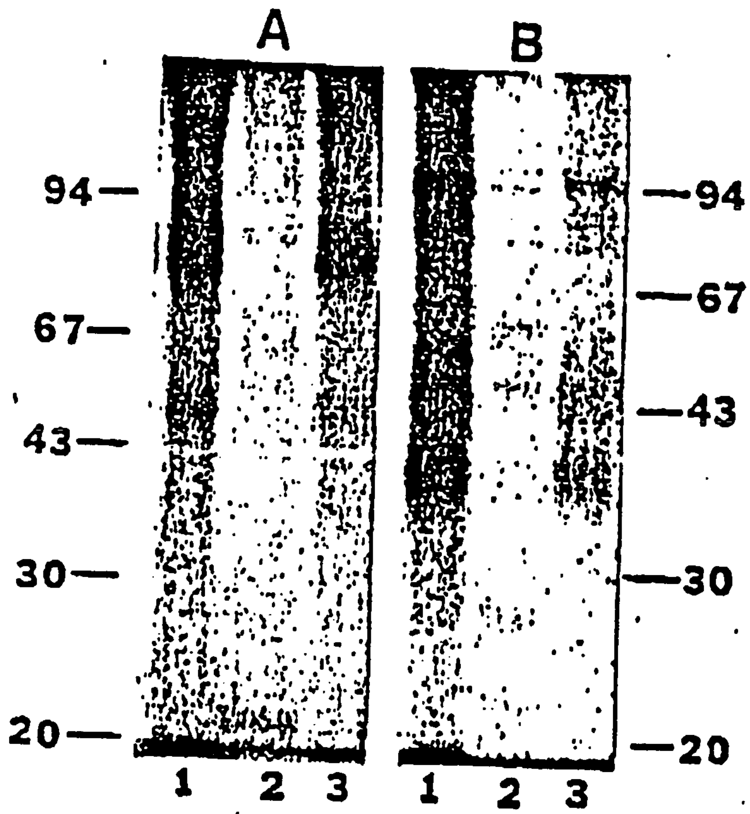

Fig. 1

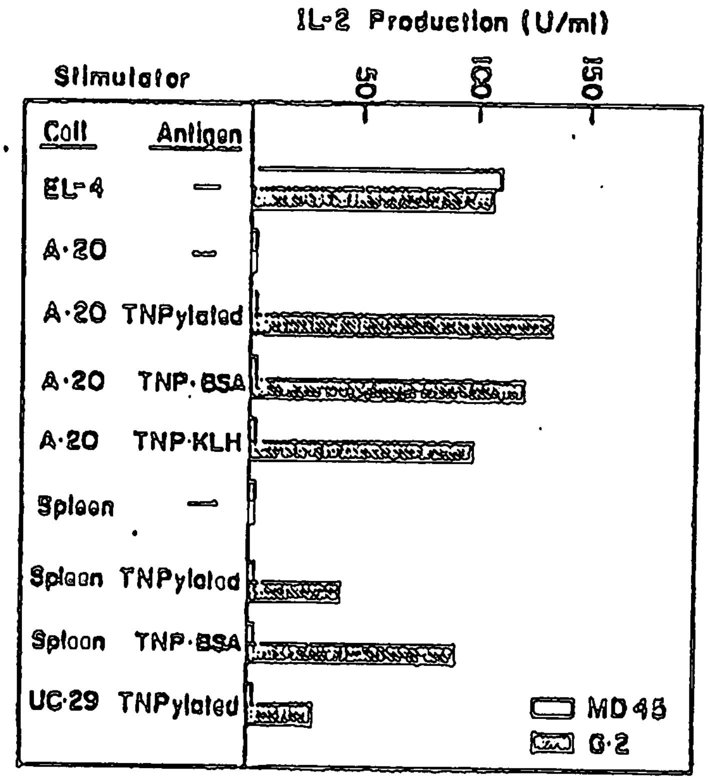
IL-2 Production (U/ml)
Stimulator
50
100
150
Cell
Antigen
EL-4
—
A·20
—
A·20
TNPylated
A·20
TNP·BSA
A·20
TNP·KLH
Spleen
—
Spleen
TNPylated
Spleen
TNP·BSA
UC·29
TNPylated
MD45
G·2

Fig. 2